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CRISPR/Cas9, a new approach to successful knockdown of ABCB1/P-glycoprotein and reversal of chemosensitivity in human epithelial ovarian cancer cell line

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ABSTRACT

Objective(s): Multidrug resistance (MDR) is a major obstacle in the successful chemotherapy of ovarian cancer. Inhibition of P-glycoprotein (P-gp), a member of ATP-binding cassette (ABC) transporters, is a well-known strategy to overcome MDR in cancer. The aim of this study was to investigate the efficiency and ability of CRISPR/Cas9 genome editing technology to knockdown ABCB1 gene expression in adriamycin resistant (A2780/ADR) ovarian cancer cell line and evaluate the sensitivity changes to doxorubicin.

Materials and Methods: Three single-guide RNAs (sgRNAs) targeting the fourth and fifth exons of human ABCB1 gene were designed in this study. Expression level of ABCB1 was detected using quantitative real time PCR (qRT-PCR) after co-transfection of all three sgRNAs into A2780/ADR cell line and subsequent antibiotic selection. Drug sensitivity to doxorubicin was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: The results showed that CRISPR/Cas9 system could significantly reduce the expression of P-gp. The dramatic decline in ABCB1 gene expression was associated with increased sensitivity of cells transfected with sgRNAs to doxorubicin.

Conclusion: Based on the results of this study, it is concluded that the CRISPR-based systems, used in the present study, effectively down-regulated the target gene and acted as an ideal and cost-effective tool for gene editing of A2780/ADR cell line resulting in restoration of nonmalignant phenotype.

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Introduction

Epithelial ovarian cancer is the sixth most common cancer and most lethal gynecological malignancy among women (1, 2). Due to the location of the ovaries within the pelvis, ovarian cancer does not represent specific symptoms in the early stages. Therefore, two thirds of the patients are diagnosed at the advanced stages and 5-year survival rate is estimated to be less than 25% (3–5). Surgery and chemotherapy are recommended as initial treatment and taxane therapy with a platinum analog is the backbone of chemotherapy (6, 7). Despite these strategies, recurrence often occurs within 2 years. These relapsing tumors often exhibit chemo-resistance towards several anti-cancer agents with various structures and mechanisms of action (3, 8). MDR in cancer is generally the result of increased expression of membrane efflux proteins which belong to the ATP-binding cassette (ABC) transporters superfamily. Consequently, cancer cells actively transfer a wide variety of pharmaceutical compounds, including chemotherapeutic agents out of the cells, resulting in reduced intracellular drug accumulation in resistant cells (9). Classical multidrug resistance to chemotherapeutic agents is attributed to the overexpression of ABCB1 gene (known as P-glycoprotein (P-gp) or MDR1) (10). It has been documented that overexpression of P-gp is the key factor for reduced chemo-sensitivity in a wide range of tumors, including ovarian cancer (11, 12). Couple of studies have indicated that several signal transduction pathways are involved in dysregulation of ABCB1 gene. According to this, it seems that targeting ABCB1, as a downstream object in multiple pathways, is the ideal choice to restore drug sensitivity in MDR cancer cells (9,13). Over the past decade, a revolutionary technology named genome editing has been developed that provides

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